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Taurine supplementation to alternative dietary proteins used in fish meal replacement enhances growth of juvenile cobia (*Rachycentron canadum*)

Angela N. Lunger [a,b], E. McLean [b], T.G. Gaylord [c], D. Kuhn [d], S.R. Craig [a,b,*]

[a] *Department of Large Animal Clinical Sciences, Va/Md Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 USA*
[b] *Virginia Tech Aquaculture Center, Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 USA*
[c] *USDA/ARS/PWA/SGPGRU, Hagerman Fish Culture Experiment Station, 3059F National Fish Hatchery Rd., Hagerman, ID 83332 USA*
[d] *Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 USA*

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Abstract

Two separate 8 week feeding trials were conducted to examine the impacts of fish meal replacement with an organically certifiable yeast-based protein source with and without supplementation of methionine, tryptophan, and taurine to diets for juvenile cobia. In the first trial, diets were formulated to contain 41% crude protein and 13% lipid, and a yeast-based protein replaced fish meal at 50 and 75% of dietary protein with and without supplemental taurine at 0.5 g/100 g dry diet. The control diet contained 100% herring fish meal. Methionine and tryptophan were added to all diets except the control to resemble the amino acid profile of fish meal. Results from this study indicated that fish fed diets supplemented with taurine exhibited significantly higher weight gain and better feed efficiencies than all other fish. Diet significantly impacted biological indices such as muscle ratio (MR), visceral somatic index (VSI), and hepatosomatic index (HSI). The 75% yeast-based protein diet without taurine returned the lowest MR values and the highest VSI and HSI values. In the second trial, diets were formulated to contain 43% crude protein and 11% lipid, with the control diet containing 100% herring fish meal and the same yeast-based protein replacing fish meal at 50, 75, and 100% of dietary protein. All diets except the control were supplemented with taurine at 0.5 g/100 g dry diet. Results from this study indicated that increasing amount of yeast-based protein led to decreased weight gains and feed efficiencies regardless of taurine supplementation. However, weight gain and feed efficiencies did increase when compared to a previous study [Lunger, A.N., McLean, E., Craig, S.R., 2007. The effects of organic protein supplementation upon growth, feed conversion and texture quality parameters in juvenile cobia (*Rachycentron canadum*). Aquaculture 264, 342–352] using identical diet formulations except for taurine supplementation. MR values tended to decrease while VSI and HSI values tended to increase with increasing fish meal replacement. It is obvious from the results from both of the present studies that taurine supplementation does have a significant impact on growth and feed efficiency of juvenile cobia when they are fed diets containing high levels of plant-based proteins as

* Corresponding author. Department of Large Animal Clinical Sciences, Va/Md Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 USA. Tel.: +1 540 231 5007; fax: +1 540 231 1676.
E-mail address: scraig@vt.edu (S.R. Craig).

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replacements for fish meal. Additionally, alternate proteins, especially those of plant and yeast-based origin can be incorporated at very high levels in diets for cobia with proper amino acid supplementation.

Keywords: Amino acid; Alternate proteins; Yeast; Quality; Fish meal replacement

1. Introduction

In aquaculture production, fish meal is typically regarded as the main protein source in diets for carnivorous fish due to its high level of protein, excellent amino acid profile which provides adequate levels of all essential amino acids, low carbohydrate level, high digestibility, and few antinutritional factors (Zhou et al., 2004). As aquaculture production continues to increase, so too has the industry's demand for fish meal. However, due to the stagnant supply of fish meal, prices will inevitably increase with demand (FAO, 2004; Lunger et al., 2007). This has amplified the need to investigate alternative protein sources. The use of plant proteins in diets for carnivorous species creates a challenge since they typically require higher levels of protein in their diet and plant proteins are less palatable. Nevertheless, several studies have shown promising results using plant-based protein sources in aquafeed formulations (Gomes et al., 1995; McGoogan and Gatlin, 1997; Fagbenro and Davies, 2001; Tidwell and Allan, 2001; Forster, 2002; Pereira and Oliva-Teles, 2003; Chou et al., 2004). Plant protein sources that have received the most interest are soybean meal and corn gluten meal due to their good amino acid profiles except for methionine which is limiting in soybean meal (El-Sayed, 1999), and lysine which is limiting in corn gluten mean (Pereira and Oliva-Teles, 2003).

Total replacement of fish meal and soybean meal with an organically certifiable yeast-based protein has been reported in diets for tilapia without impacting weight gain (Craig and McLean, 2005). In a previous study using cobia, a carnivorous species, the same yeast-based protein was able to replace 25% of the fish meal protein without impacting growth parameters (Lunger et al., 2006). Further dietary inclusion rates for this protein might be problematic however due to amino acid imbalances (Craig and McLean, 2006).

Methionine is an essential amino acid required by fish for normal growth and metabolic functions (NRC, 1993; Luo et al., 2005). Since many plant proteins are deficient in methionine, it is typically the first limiting amino acid in diets which replace fish meal with large amounts of plant protein. Methionine deficiencies result in reduced growth rates, feed efficiency and survival (Goff and Gatlin, 2004) such that in diets containing high levels of plant proteins, methionine supplementation must be utilized so that growth is not compromised (Jackson and Capper, 1982, Murai et al., 1982, Takagi et al., 2001).

Taurine is not considered to be an essential amino acid because it can be synthesized by fish. As in mammals, Yokoyama et al. (1997) demonstrated that rainbow trout synthesized taurine from cysteine. In the mammalian system, taurine is synthesized through many enzymatic reactions; but the enzyme L-cysteine-sulphinate decarboxylase appears to be rate-limiting (Jacobsen and Smith, 1968). Activity of this enzyme varies in fish depending upon species and size. For example, in the yellowtail, as well in bluefin and skipjack tunas, L-cysteinesulphinate decarboxylase activity is not present, whereas in Japanese flounder it expresses only low activity (Yokoyama et al., 2001).

Taurine is typically found in relatively high concentrations in fish meal and animal by-products but is almost non-existent in plant meals. Even when all essential amino acid requirements are met in plant-based diets for carnivorous fish, growth still is often reduced when compared to fish meal-based diets (Gaylord et al., 2006). Therefore, taurine supplementation may be required for plant-based diets and indeed, dietary taurine additions improve weight gain and feed efficiency in olive flounder (Park et al., 2002; Kim et al., 2005a), as well as in rainbow trout (Gaylord et al., 2006). Based on this information, the current studies were undertaken to examine whether higher levels of fish meal could be replaced in cobia diets utilizing yeast-based proteins and specific amino acid supplementation including methionine, tryptophan and taurine.

2. Materials and methods

2.1. Experiment 1

2.1.1. Experimental system and husbandry

All studies were undertaken using a recirculating aquaculture system. The 3400 l recirculation configuration (flow rate = 4 l/min-aquaria) was comprised of 24,

110 l glass aquaria (15 aquaria dedicated to this study) serviced with a 750 l (200 gal) KMT-based (Kaldnes Miljøteknologi, Tønsberg, Norway) fluidized bed biofilter, a bubble-bead filter (Aquaculture Technologies Inc., Metaire, LA) for solids removal, a protein skimmer (R&B Aquatics, Waring, TX), and a 40 W UV sterilizer (Aquatic Ecosystems, Apopka, FL). The fluidized bed was oxygenated using diffusion air lines connected to a 1 hp Sweetwater remote drive regenerative blower (Aquatic Ecosystems, Apopka, FL). During the feeding trial, water temperature (mean ± S.D.: 26.5 ± 1.5 °C) and pH (7.82 ± 0.27) were monitored 3 times a week using a Hanna Instrument 9024 pH meter (Aquatic Ecosystems, Apopka, FL). Dissolved oxygen (5.33 ± 0.98 mg/l) and total ammonia nitrogen (0.49 ± 0.24 mg/l) were also measured three times a week using an YSI 85 Series dissolved oxygen meter (YSI Inc., Yellow Springs, OH) and by spectrophotometric analysis (Hach Inc., Loveland, CO), respectively. Nitrite (0.59 ± 0.49 mg/l) and nitrate (61 ± 10 mg/l) levels were quantified once a week by spectrophotometric analysis. Salinity was monitored and maintained near 17 ppt (17.5 ± 1.6 ppt) using Crystal Sea synthetic sea salt (Marineland, Baltimore, MD). A photoperiod using phosphorescent tubes positioned 1.8 m above the system was implemented using a 12 h photophase–scotophase cycle using an automated timer with a half hour dusk/dawn period.

Juvenile cobia (*Rachycentron canadum*) were supplied by the Virginia Seafood Agricultural Research and Extension Center (Hampton, VA, USA). Fish were transported to the Virginia Tech Aquaculture Center (VTAC, Blacksburg, VA) and were acclimated and maintained in eight 500 l tanks for approximately 2 weeks. Upon commencement of the experiment, seven juvenile cobia, (mean individual weight with one standard deviation, 9.8 ± 0.3 g), were randomly placed into each of 15 experimental tanks. Fish were hand-fed the experimental diets (Table 1) twice daily, at 09.00 and 16.00 h. The ration was divided equally between the two feedings. Fish were fed 10, 10, 10, 8, 7, 6, 5, and 4% body weight per day, during weeks 1, 2, 3, 4, 5, 6, 7, and 8, respectively. This maintained a level of apparent satiation without overfeeding. Tanks were group weighed weekly to adjust the feeding rates and monitor growth performance.

2.1.2. Diets

Experimental feeds were produced as summarized in Table 1. The control diet was composed of 100% herring fish meal, with the remaining diets produced by replacing fish meal with NuPro® (Alltech Inc., Nicholasville, KY) at 50 and 75 g/100 g on a nitrogenous basis. Methionine (0.3 g/100 g dry diet) and tryptophan (0.2 g/100 g dry diet) were added to these diets based upon amino acid analysis of previous diets utilizing NuPro® and similar replacement levels. Two additional diets were manufactured by adding taurine (0.5 g/100 g dry diet) at each fish meal replacement level. Each feed treatment was performed in triplicate. The dry dietary components of the diet were first mixed in a Patterson-Kelley twin shell® Batch V-mixer (Patterson-Kelley Co. Inc., East Stroudsburg, PA) for 20 min then transferred into a Hobart D300 Floor Mixer (Hobart Co., Troy, OH). Menhaden fish oil was added and mixed for an additional 5 min. The amount of distilled water required for pelleting (20–40 g/100 g of feed weight) was then added to the mixture and mixed until a pebble-like consistency was achieved. The mixture was then pressure pelleted using an appropriate die to provide pellets of suitable size for the fish. After air-drying, feed was frozen at −20 °C until needed. To determine dry matter, duplicate samples from each feed were heated at 135 °C for 2 h in a gravity oven (Blue M Electric, Blue

Table 1
Composition of diets for trial 1 (g/100 g on a dry matter basis): 41% crude protein, 13% lipid and 300 kcals/100 g

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
	100:0	50:50	50:50+ Tau	25:75	25:75+ Tau
Herring meal [a]	58.6	29.3	29.3	14.6	14.6
Yeast protein [b]	0.0	40.0	40.0	59.9	59.9
Dextrin [c]	4.9	4.9	4.9	4.9	4.9
Menhaden Fish oil [d]	7.1	9.3	9.3	10.3	10.3
Mineral mix [e]	4.0	4.0	4.0	4.0	4.0
Vitamin mix [f]	3.0	3.0	3.0	3.0	3.0
CMC [c]	1.0	1.0	1.0	1.0	1.0
$CaPO_4$ [g]	0.0	1.0	1.0	1.0	1.0
Methionine [g]	0.0	0.3	0.3	0.3	0.3
Tryptophan [g]	0.0	0.2	0.2	0.2	0.2
Taurine [g]	0.0	0.0	0.5	0.0	0.5
Cellufill [c]	21.4	7.0	6.5	0.8	0.3
Crude protein [h]	41.6	40.8	41.1	41.3	41.6
Crude lipid [h]	10.6	10.4	10.6	10.5	11.3
Taurine (% of diet) [h]	0.41	0.24	0.63	0.13	0.55
Available energy [i] (kcals/100 g diet)	300	300	300	300	300

[a] International Proteins Corporation, Minneapolis, MN, USA.
[b] Alltech Incorporated, Nicholasville, KY, USA.
[c] US Biochemical Corporation, Cleveland, Ohio, USA.
[d] Omega oils, Reedville, VA, USA.
[e] ICN Corporation, Costa Mesa, CA, USA.
[f] See Moon and Gatlin (1991).
[g] Aldrich-Sigma, St. Louis, MO
[h] Analyzed.
[i] Calculated.

Island, IL, USA). Prior to use as feed, small quantities were thawed and refrigerated.

2.1.3. Data acquisition

At the end of the feeding trial, three fish from each tank ($N=9$ treatment^{-1}) were euthanized by an overdose of clove oil (3 mg/l; Sigma-Aldrich, St. Louis, MO) and bled via caudal venipuncture for measurement of packed cell volume (PCV), plasma protein levels, and plasma amino acid analysis. Fish were measured for length and weight and weight gain, feed efficiency ratio values, survival, visceral somatic index (VSI), hepatosomatic index (HSI), and muscle ratio (MR) were calculated. Muscle and liver samples also were collected for proximate analysis, including crude protein, total lipid, dry matter and ash (AOAC, 1994). Plasma taurine, methionine and tryptophan levels were quantified according to a modified method of Petritis et al. (1999) using an Agilent 1100 series LCMSD Trap (Agilent Technologies, Palo Alto, CA). Injection volume was 15 µl. Separation was carried out on a gradient at a flow rate of 0.6 ml/min. Mobile phase A was 0.1% heptafluorobutyric acid (Sigma-Aldrich Co., St. Louis, MO) and mobile phase B was 100% acetonitrile. Samples were injected on a 5 µm, 125×4 mm Purospher RP-18E column with 4×4 mm precolumn (Agilent Technologies, Palo Alto, CA) using an automated injection sequence. Mass spectrometry was performed in positive ion mode with nebulizer pressure at 45 psi, drying gas flow at 9.5 l/min, drying gas temperature at 350 °C, and capillary voltage at 3500 V. Prior to injection, 60 µl of plasma from each of three fish per tank was pooled, for a total of 180 µl of plasma per tank. The pooled plasmas were then divided into three 50 µl aliquots. Each aliquot of pooled plasma was diluted with 50 µl of mobile phase A. Plasma proteins were then precipitated out with 300 µl of 100% methanol, followed by incubation for 10 min at room temperature and centrifugation at 17,500 ×g at 4 °C for 5 min (Piraud et al., 2005). Next, the resulting supernatants were transferred directly to autosampler vials with inserts and injected. Amino acid concentrations were calculated using QuantAnalysis software (Version 1.6, Build 121, Bruker Daltonik, Billerica, MA).

2.1.4. Statistical analyses

All data were subjected to analysis of variance procedures utilizing SAS 9.1 (SAS, Cary, NC, USA). When appropriate, a post-hoc test (Duncan's multiple range test) was used to check for significant differences ($\alpha<0.05$) between the means.

2.2. Experiment 2

2.2.1. Experimental system and husbandry

The same experimental system was used for this study but only 12 aquaria were required. Water quality parameters were within similar ranges as recorded during the first study. The 12 aquaria were initially stocked with seven fish each (mean individual weight ~28 g per fish) and were hand-fed as previously described. Cobia were fed between 8 and 4% body weight to maintain apparent satiation. Tanks were group weighed weekly to adjust the feeding rates and monitor growth performance.

2.2.2. Diets

Diets were manufactured in an identical manner to those employed in the first experiment. A 100% herring fish meal diet was used as the control and three other experimental diets were formulated. The yeast-based, organically certified product NuPro® (Alltech Inc., Nicholasville, KY, USA) was used to replace 50, 75, and 100% fish meal in diets 2, 3, and 4, respectively. Taurine was also added to each diet at 0.5 g/100 g dry diet (Table 2).

Table 2
Composition of diets for trial 2 (g/100 g on a dry matter basis) formulated to provide 43% crude protein and 11% lipid (dry matter basis)

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
	100:0	50:50+ Tau	25:75+ Tau	0:100+ Tau
Herring meal [a]	57.2	28.6	14.3	0.0
Yeast protein [b]	0.0	41.9	62.9	83.8
Dextrin [c]	9.5	5.6	4.8	3.4
Menhaden Fish oil [d]	6.0	8.1	8.5	8.3
Mineral mix [e]	4.0	4.0	4.0	4.0
Vitamin mix [f]	3.0	3.0	3.0	3.0
CMC [c]	1.0	1.0	1.0	1.0
$CaPO_4$ [g]	0.0	1.0	1.0	1.0
Taurine [g]	0.0	0.5	0.5	0.5
Cellufil [c]	19.3	6.3	0.0	0.0
Crude protein [h]	43.6	44.0	44.1	43.9
Crude lipid [h]	9.8	10.4	9.8	9.7
Available energy [i] (kcal/100 g diet)	309	293	290	285

[a] International Proteins Corporation, Minneapolis, MN, USA.
[b] Alltech Incorporation, Nicholasville, KY, USA.
[c] US Biochemical Corporation, Cleveland, Ohio, USA.
[d] Omega oils, Reedville, VA, USA.
[e] ICN Corporation, Costa Mesa, CA, USA.
[f] See Moon and Gatlin (1991).
[g] Aldrich-Sigma, St. Louis, MO, USA.
[h] Analyzed.
[i] Calculated.

Table 3
Weight gain, specific growth rates (SGR), feed efficiency ratio values (FE), and survival percentages of juvenile cobia in trial 1 fed diets in which fish meal was replaced by a yeast-based protein[1]

Diet (fish meal:yeast)	Weight gain[2] (% increase)	SGR[3]	FE[4]	Survival %
100:0	981[b]	4.17[a]	0.39[b]	95[a]
50:50	753[c]	3.49[b]	0.41[b]	62[b]
50:50+Tau	1350[a]	4.47[a]	0.55[a]	66[ab]
25:75	337[d]	2.46[c]	0.25[c]	33[c]
25:75+Tau	1249[a]	4.61[a]	0.53[a]	90[ab]
Pooled SE	59.5	0.16	0.02	8.84
$P<F$	<0.0001	<0.0001	<0.0001	0.0038

[1]Means of 3 tanks treatment^{-1}. Means with different superscripts in the same column differed significantly ($P<0.05$).
[2]Weight gain=(final tank weight−initial weight)/initial tank weight.
[3]SGR=(ln final wt.−ln initial wt.)/49 days.
[4]FE=g gained/g fed.

2.2.3. Analysis

Data acquisition and statistical analyses were undertaken as described previously.

3. Results

3.1. Experiment 1

Weight gain (% increase from initial weight) ranged from 337 to 1350% and was significantly affected by diet (Table 3). Cobia that were fed the 50 and 75% yeast-based diets supplemented with taurine (Diets 3 and 5, respectively) had significantly higher ($P<0.0001$) weight gains than the control diet (Diet 1) and the 50 and 75% yeast-based diets without taurine supplementation (Diets 2 and 4, respectively). Fish fed diet 4 (75% yeast-based protein without taurine) had significantly poorer growth than all other fish. Specific growth rates (SGR) ranged from 2.46 to 4.61 and also were significantly impacted by diet (Table 3). SGRs for cobia fed the control diet (Diet 1) and the 50 and 75% yeast-based diets with taurine were similar whereas fish fed the 50% and 75% yeast-based diets without supplemental taurine exhibited lower SGR values. Fish fed the 75% yeast-based protein diet without taurine recorded the lowest SGRs in the first feeding trial ($P<0.0001$). Feed efficiency (FE) ratio values ranged from 0.25 to 0.55 (Table 3) and followed a similar trend as weight gain. Cobia fed diets supplemented with taurine had significantly higher FE values than the control. FE values for the control diet and the 50% yeast protein diet without taurine were the same, but cobia fed the 75% yeast-based diet without taurine had significantly lower FE values ($P<0.0001$). Survival was variable with most mortalities resulting from an air line failure in week 6 of the trial. It is interesting to note that fish fed the diets containing supplemental taurine more readily survived this mechanical failure.

Muscle ratios ranged from 17.6 to 30.0% and also followed a similar trend as weight gain. Fish fed control, 50% and 75% yeast-based diets with taurine expressed similar muscle ratios (Table 4). Cobia fed 50% replacement protein without taurine had similar muscle ratio values to control and 75% replacement plus taurine diets, but were lower than that recorded for fish fed 50% yeast-based protein plus taurine. Fish fed 75% replacement protein without taurine once again had lower muscle ratio values than all others ($P<0.0001$; Table 4). Visceral somatic index (VSI) ranged from 9.0 to 14.1% (Table 4) with significant impact of diet. Fish fed the control diet had lower VSI values than all other

Table 4
Biological indices including the muscle ratio (MR), visceral somatic index (VSI), and hepatosomatic index (HSI) of juvenile cobia in trial 1 fed diets in which fish meal was replaced with a yeast-based protein[1]

Diet (fish meal:yeast)	MR[2]	VSI[3]	HSI[4]
100:0 Control	27.2[ab]	9.0[d]	1.59[d]
50:50	24.4[b]	11.5[b]	4.03[b]
50:50+Tau	30.0[a]	10.0[c]	3.11[c]
25:75	17.6[c]	14.1[a]	5.07[a]
25:75+Tau	27.0[ab]	11.6[b]	3.65[bc]
Pooled SE	1.72	0.59	0.32
$P<F$	<0.0001	<0.0001	<0.0001

[1]Means of 3 tanks treatment^{-1}. Means with different superscripts in the same column differed significantly ($P<0.05$).
[2]MR=muscle weight*100/body weight.
[3]VSI=VSI weight*100/body weight.
[4]HSI=HSI weight*100/body weight.

Table 5
Muscle protein, muscle lipid, muscle dry matter and ash and liver lipid of juvenile cobia in trial 1 fed diets in which fish meal was replaced with a yeast-based protein[1]

Diet (fish meal:yeast)	Muscle protein[1]	Muscle lipid[2]	Muscle dry matter[1]	Muscle ash[1]	Liver lipid[2]
100:0 Control	83.1[a]	2.58[a]	23.12[c]	6.47[b]	18.14[b]
50:50	80.3[ab]	2.48[a]	24.28[b]	6.51[b]	30.80[a]
50:50+Tau	78.9[b]	2.63[a]	25.40[a]	5.83[bc]	22.68[ab]
25:75	83.1[a]	1.42[b]	21.99[d]	7.52[a]	15.89[b]
25:75+Tau	78.4[b]	3.02[a]	25.29[a]	5.50[c]	20.64[b]
Pooled SE	1.97	0.38	0.58	0.42	3.41
$P<F$	0.0151	0.0177	<0.0001	<0.0001	0.0382

[1]Means of 3 fish per tank ($N=9$ treatment^{-1}). Means with different superscripts in the same column differed significantly ($P<0.05$). Values are presented on a dry matter basis.
[2]Means of 1 fish per tank ($N=3$ treatment^{-1}). Means with different superscripts in the same column differed significantly ($P<0.05$). Values are presented on a wet weight basis.

Table 6
Plasma amino acid values of juvenile cobia in trial 1 fed diets in which fish meal was replaced with a yeast-based protein[1]

Diet (fish meal:yeast)	Taurine	Methionine	Tryptophan
100:0 Control	555.5[a]	47.8	10.4
50:50	26.1[c]	42.5	8.7
50:50+Tau	221.8[b]	47.1	12.0
25:75	5.4[c]	42.3	9.6
25:75+Tau	223.3[b]	32.9	9.3
Pooled SE	7.51	3.18	1.14
$P<F$	<0.0001	0.0513	0.3358

[1]Units are expressed as nmol/ml plasma.

treatments whereas the highest VSI was observed in fish fed 75% protein replacement without taurine ($P<0.0001$). Hepatosomatic index ranged from 1.59 for the control diet to 5.07 for the 75% protein replacement diet without taurine (Table 4). HSI values followed the same trend as observed for VSI (Table 4).

Muscle protein ranged from 78 to 83 g/100 g dry weight and was only slightly impacted by diet (Table 5). Fish fed the alternate protein with taurine expressed lowest muscle protein content, which was the same as cobia provided with 50% yeast protein without taurine. Muscle lipid ranged from 1.42 to 3.02 g/100 g wet weight (Table 5) with cobia fed the 75% yeast-based protein diet without taurine expressing lowest levels ($P<0.02$). Liver lipid ranged from 15.9 to 30.8 g/100 g wet weight and was significantly affected by diet (Table 5). Muscle dry matter ranged from 22.0 to 25.4 g/100 g and differed across all groups, whereas muscle ash ranged from 5.5 to 7.5 g/100 g (Table 5). Fish fed the diet containing 75% yeast protein without taurine supplementation had significantly higher muscle ash content while fish fed both diets that were supplemented with taurine had significantly lower ash contents than all other fish.

Table 8
Biological indices including the muscle ratio (MR), visceral somatic index (VSI), and hepatosomatic index (HSI) of juvenile cobia in trial 2 fed diets in which fish meal was replaced with yeast-based protein[1]

Diet (fish meal:yeast)	MR[2]	VSI[3]	HSI[4]
100:0 Control	27.9[a]	8.2[c]	1.64[c]
50:50	24.0[ab]	9.1[bc]	2.24[b]
25:75	21.9[b]	9.8[b]	2.60[b]
0:100	22.4[b]	10.8[a]	3.31[a]
Pooled SE	2.40	0.54	0.28
$P<F$	0.0180	<0.0001	<0.0001

[1]Means of 3 tanks treatment^{-1}. Means with different superscripts in the same column differed significantly ($P<0.05$).
[2]MR=muscle weight * 100/body weight.
[3]VSI=VSI weight * 100/body weight.
[4]HSI=HSI weight * 100/body weight.

Plasma amino acid values for taurine (nmol/ml) ranged from 5 to 555 (Table 6). Fish fed the control diet had significantly higher plasma taurine levels. Fish fed the diets supplemented with taurine had intermediate plasma taurine levels (222 and 223 nmol/ml) while fish fed the unsupplemented diets had the lowest plasma taurine levels (26 and 5 nmol/ml). Plasma methionine and tryptophan levels ranged from 33 to 48 nmol/ml and 8.7 to 12.0 nmol/ml, respectively (Table 6), and did not differ significantly with respect to diet.

3.2. Experiment 2

All diets were supplemented with taurine except for the 100% herring fish meal control. Weight gain ranged from 280 to 524% increase and was significantly affected by diet (Table 7). Fish fed the control and 50/

Table 7
Weight gain, specific growth rates (SGR), feed efficiency ratio values (FE), and survival percentages of juvenile cobia in trial 2 fed diets in which fish meal was replaced with a yeast-based protein[1]

Diet (fish meal:yeast)	Weight gain[2] (% increase)	SGR[3]	FE[4]	Survival
100/0 Control	524[a]	3.27[a]	0.51[a]	100
50/50	516[ab]	3.24[a]	0.45[b]	100
25/75	430[b]	2.98[a]	0.46[b]	100
0/100	280[c]	2.38[b]	0.35[c]	95
Pooled SE	27.16	0.09	0.02	2.33
$P<F$	0.0007	0.0004	0.0008	0.4411

[1]Means of 3 tanks treatment^{-1}. Means with different superscripts in the same column differed significantly ($P<0.05$).
[2]Weight gain=(final tank weight−initial weight)/initial tank weight.
[3]SGR=(ln final wt.−ln initial wt.)/days of study.
[4]FE=g gained/g fed.

Table 9
Muscle protein, muscle lipid, muscle dry matter and ash and liver lipid of juvenile cobia in trial 2 fed diets in which fish meal was replaced with a yeast-based protein

Diet (fish meal:yeast)	Muscle protein[1]	Muscle lipid[2]	Muscle dry matter[1]	Muscle ash[1]	Liver lipid[2]
100:0 Control	85.3	1.48	22.86	6.02	5.91
50:50	85.5	1.13	22.73	5.97	9.47
25:75	84.7	1.78	23.20	5.69	10.22
0:100	86.6	1.31	22.75	5.77	4.11
Pooled SE	1.59	0.30	0.44	0.16	1.45
$P<F$	0.8560	0.5144	0.8599	0.4608	0.0510

[1]Means of 1 fish per tank ($N=3$ treatment^{-1}). Means with different superscripts in the same column differed significantly ($P<0.05$). Values are presented on a dry matter basis.
[2] Means of 1 fish per tank ($N=3$ treatment^{-1}). Means with different superscripts in the same column differed significantly ($P<0.05$). Values are presented on a wet weight basis.

Table 10
Plasma amino acid values of juvenile cobia in trial 2 fed diets in which fish meal was replaced with a yeast-based protein[1]

Diet (fish meal:yeast)	Taurine	Methionine	Tryptophan
100:0 Control	306.9	87.2[a]	15.5
50:50	382.0	76.4[a]	13.5
25:75	336.8	63.5[b]	16.0
0:100	248.6	38.9[c]	13.2
Pooled SE	29.28	3.55	1.47
$P<F$	0.0635	<0.0001	0.4693

[1]Units are expressed as nmol/ml plasma.

50 diets expressed identical and highest growth increases ($P>0.05$). As the percentage of replacement protein increased, weight gain decreased and fish fed 100% replacement returned the lowest overall weight increase ($P=0.0010$). Specific growth rates (SGR) and feed efficiency (FE) ratio values followed the same trend as weight gain, decreasing with increasing level of fish meal replacement (Table 7). Fish fed the control diet however, had significantly higher FE values than all other diets ($P=0.0008$).

Muscle ratio (MR) ranged from 21.9 to 27.9% with the highest value being expressed by control diet fed fish (Table 8). The visceral somatic index (VSI) ranged from 8.2 to 10.8% and was significantly affected by diet (Table 8). VSI tended to increase with increasing percentage of fish meal replacement with fish fed 100% replacement expressing highest VSI ($P<0.0001$). Hepatosomatic index (HSI) ranged from 1.64 to 3.31 and also was significantly affected by diet. As with VSI, animals maintained on the 100% fish meal replacement diet had highest HSI values (Table 8).

No between diet differences were recorded for muscle protein (85–87% dry weight), muscle lipid (1.1–1.8% wet weight), liver lipid (4.1–10.2% wet weight), dry matter (22.7–23.2%) or ash levels (5.7–6.0%; Table 9).

Plasma amino acid values for taurine and tryptophan (nmol/ml) ranged from 249 to 382 and 13.2 to 16.0, respectively (Table 10), and did not differ significantly with respect to diet. Plasma methionine levels were significantly impacted by fish meal replacement with levels ranging from 39 to 87 nmol/ml (Table 10). Fish fed the control and the 50/50 diets had similar plasma methionine levels (87 and 76 nmol/ml, respectively) which then decreased significantly with increasing yeast inclusion rates (64 nmol/ml for the 25/75 diet and 39 nmol/ml for 100% yeast diet).

4. Discussion

The production of fish meal has remained relatively stable over the past 15 years, and this situation is unlikely to improve (FAO, 2004; Lunger et al., 2007). Indeed it has been suggested that the availability of fish meal will decline in the future such that fish meal can no longer be considered as a sustainable protein source for aquafeeds (Craig and McLean, 2006). Accordingly, alternate proteins are needed to replace fish meal especially in diets for carnivorous species. Plant proteins are probably the most widely used alternative to fish meal, but they express problems including lower crude protein levels, palatability issues, amino acid deficiencies and the occurrence of antinutritional factors such as trypsin inhibitors (Francis et al., 2001). Taurine is not considered to be an essential amino acid for fish, but is a free amino acid that is present in large quantities in various tissues of marine fish (Park et al., 2002). In mammals, taurine plays important roles in osmoregulation, bile acid conjugation, membrane stabilization, hormone release, modulation of neurotransmitters, and antioxidation (Sturman, 1988; Huxtable, 1992). It is also a required amino acid in diets for cats due to their low cysteinesulfinate decarboxylase activity (Knopf et al., 1978). Carnivorous fish in the wild consume relatively large quantities of taurine since it is highly abundant in animal tissues, but this does not apply when diets contain large amounts of plant protein sources, which are naturally low in taurine. Therefore, it may be necessary to supplement these diets with taurine and other amino acids to maintain production characteristics. In both of our feeding trials we added taurine at 0.5 g/100 g dry weight to diets that contained high levels of a yeast-based protein source with the result that fish fed diets supplemented with taurine gained significantly more weight and generally returned better feed efficiency ratios than animals fed the control diet. Growth rates and feed efficiency ratios also have been improved with taurine supplementation in species such as Japanese flounder (Park et al., 2002; Kim et al., 2003, 2005a,b), European sea bass (Martinez et al., 2004), yellowtail (Matsunari et al., 2005), rainbow trout (Gaylord et al., 2006), and black tiger shrimp (Shiau and Chou, 1994). In our second trial, weight gain, specific growth rate, and feed efficiency ratios were also improved with taurine supplementation, but all measurements tended to decrease with increasing level of dietary fish meal replacement. The same trend was observed when the same yeast-based protein was substituted at identical levels without taurine (Lunger et al., 2006). However, the diet containing 100% of the yeast-based protein source in Lunger et al. (2006) returned only 86% increase from initial weight utilizing 11 g fish, whereas in the present study, the diet containing 100% of the yeast-based protein source

returned over 280% increase from initial weight starting with much larger fish (28 g initial weight). In our previous study feed intake of fish fed diets containing high levels of yeast protein was low. The diet containing 100% of the yeast-based protein source in Lunger et al. (2006) was repeatedly spit out by the cobia, whereas the same diet with taurine addition in the present trial was readily and eagerly consumed by the juvenile cobia, indicating no palatability issues. It has been reported that taurine can act as a feed attractant and European sea bass fry were observed to preferentially consume a diet supplemented with 0.2% taurine (Martinez et al., 2004). Taurine supplementation of juvenile cobia diets may have made the diets more palatable, thus increasing feed intake and subsequently weight gain.

Plasma amino acid analysis revealed no significant differences in methionine and tryptophan levels when these amino acids were supplemented in the diets in the first experiment. However, significant differences were observed when taurine was supplemented. Although these levels did not rise to the levels observed in the control diet which contained 100% herring fish meal, they were significantly elevated over those unsupplemented with taurine and significantly improved weight gain. Whether this result was solely due to metabolic actions of taurine (i.e. possible essentiality of taurine) or simply the impacts of increased feed intake is questionable. Plasma taurine levels in the second trial were similar to those observed in the supplemented diets in the first trial. Taurine supplementation in the second trial enhanced weight gain in fish fed the 50/50 diet equal to that of the control however, it did not have similar impacts to diets containing greater than 50% of the yeast product. In this trial, methionine was not supplemented yet the plasma data revealed higher levels than those observed in the first trial, indicating a potential deficiency in another essential nutrient, possibly lysine. While there is a correlation between plasma amino acid values and requirement levels (Kaushik, 1979; Wilson, 1994), few studies have successfully utilized this technique for accurate amino acid requirement values (Wilson and Halver, 1986). Additionally, plasma amino acid values are difficult to compare between studies due to differences in absorption rates due to experimental conditions and blood sampling times (Karlsson et al., 2006).

Evidence from the present studies indicates that taurine is conditionally indispensable when cobia are fed diets containing high levels of plant-based protein sources. Martinez et al. (2004) reported that sea bass may require dietary taurine supplementation under certain feeding practices as well. Rainbow trout that were fed a plant-based diet required taurine supplemented at 5 g/kg dry diet in order to keep up with the growth of fish fed a fish meal-based control diet (Gaylord et al., 2006). Kim et al. (2003) reported that juvenile olive flounder required taurine supplementation, whereas fingerling olive flounder did not. Optimal levels of taurine supplementation were suggested to be 15 mg/g (Kim et al., 2005b) or 15–20 mg/g (Park et al., 2001). These results when taken together, indicate that taurine supplementation is necessary for carnivorous fish species when fed diets with alternate protein sources. This could be particularly true for marine species since taurine plays a critical role in osmoregulation and typically comprises more than 50% of the free amino acid pool (Lombardini et al., 1979). Fish raised in sea water may have a greater demand for dietary taurine than fish held in fresh water and a fish's ability to convert cysteine to taurine may be based on their environmental salinity requirements (Gaylord et al., 2006). Therefore, the osmotic stabilization provided by taurine may be related to its effects on growth and the fact that supplementation to diets improves growth in numerous species (Kim et al., 2003). Some species may be unable or poor at synthesizing taurine *de novo* from cysteine. This result may be due to the activity level of L-cysteinesulphinate decarboxylase, which in turn might be influenced by the natural feeding habits of a particular species or previous feeding history (Gaylord et al., 2006). Carnivorous fish therefore, may be less able to synthesize taurine due to their naturally high intake while herbivorous/omnivorous fish may be more capable of such synthesis due to the paucity of taurine in their diets. Species with rapid growth rates, such as cobia, may also experience an increased demand on the *de novo* synthesis of taurine which cannot be met, especially when fish meal is replaced in diets by plant protein sources devoid of taurine. When a non-essential amino acid, such as taurine, is added to diets, it may be possible to conserve essential amino acids as well (Cowey, 1994), which could lead to improved growth rates. In both trials diets were fed to fish containing 50 and 75% yeast-based protein supplemented with taurine, methionine and tryptophan, methionine and tryptophan alone, or taurine alone. Of these, the best performance was recorded for the 50 and 75% replacement levels supplemented with all three amino acids followed by the diets supplemented with taurine alone. The diets supplemented with methionine and tryptophan alone resulted in inferior weight gains compared to these other diets. A reason for these results could be that when taurine was supplemented alone, its presence allowed cobia to conserve the essential amino acids (methionine

and tryptophan) that were present in the unsupplemented diets thus improving growth rates.

Due to the wide range of biological impacts associated with taurine, including anticonvulsant activity, muscle membrane stabilization, bile salt synthesis, cell proliferation and viability and antioxidant activities to name a few (Huxtable, 1992), reasons for improved growth rates observed in the present study with the addition of taurine are purely speculation. This particular area of research is very limited and results such as these certainly warrant future investigations. It is obvious from the results of both of the present studies that taurine supplementation does have a significant impact on growth and feed efficiency of juvenile cobia when they are fed diets containing high levels of plant-based alternate protein sources as replacements for fish meal. These findings could dramatically change the amount and types of alternate proteins that can be effectively incorporated into diets for juvenile cobia and decrease the industries reliance on fish meal supplies. The results from this study also magnify the importance of quantitative amino acid requirements for cobia, many of which are presently undetermined.

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